

Rapid Separations with High Efficiency Using Regis' Chiral Stationary Phases in Supercritical Fluid Chiral Chromatography (SFC)

Terry Berger¹, Jelena Kocergin², Ted Szczerba²

¹Aurora SFC Systems, Inc., Redwood City, CA,

²Regis Technologies, Inc., Morton Grove, IL 60053, USA



Abstract

Chiral method development and chiral preparative separations have been the main contributors to the popularity of supercritical fluid chromatography (SFC). The low viscosity and high diffusivity of the mobile phase in SFC allows for low pressure drops and fast chromatography making it an attractive alternative to LC, especially for pharmaceutical applications. SFC is often 3-5 times faster than HPLC and often produces higher efficiency. SFC is now regarded as a standard separation support tool for chemical synthesis and development.

A number of factors determine the success of an SFC separation. Perhaps the most important factor is the performance of the chiral stationary phase (CSP). Several CSP's will be investigated for their ability to generate fast separations and their capabilities of scaling up to preparative chromatography.

Introduction

This study evaluates the RegisPack[®], RegisCell[®] and Whelk-O[®] 1 chiral columns for their suitability in analytical and preparative SFC. The RegisPack[®] and RegisCell[®] (polysaccharide based CSP's) are coated with tris-(3,5-dimethylphenyl) carbamate of amylose and tris-(3,5-dimethylphenyl) carbamate of cellulose respectively. The Whelk-O[®] 1 (a Pirkle-Type CSP) is based on 1-(3,5-Dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene.

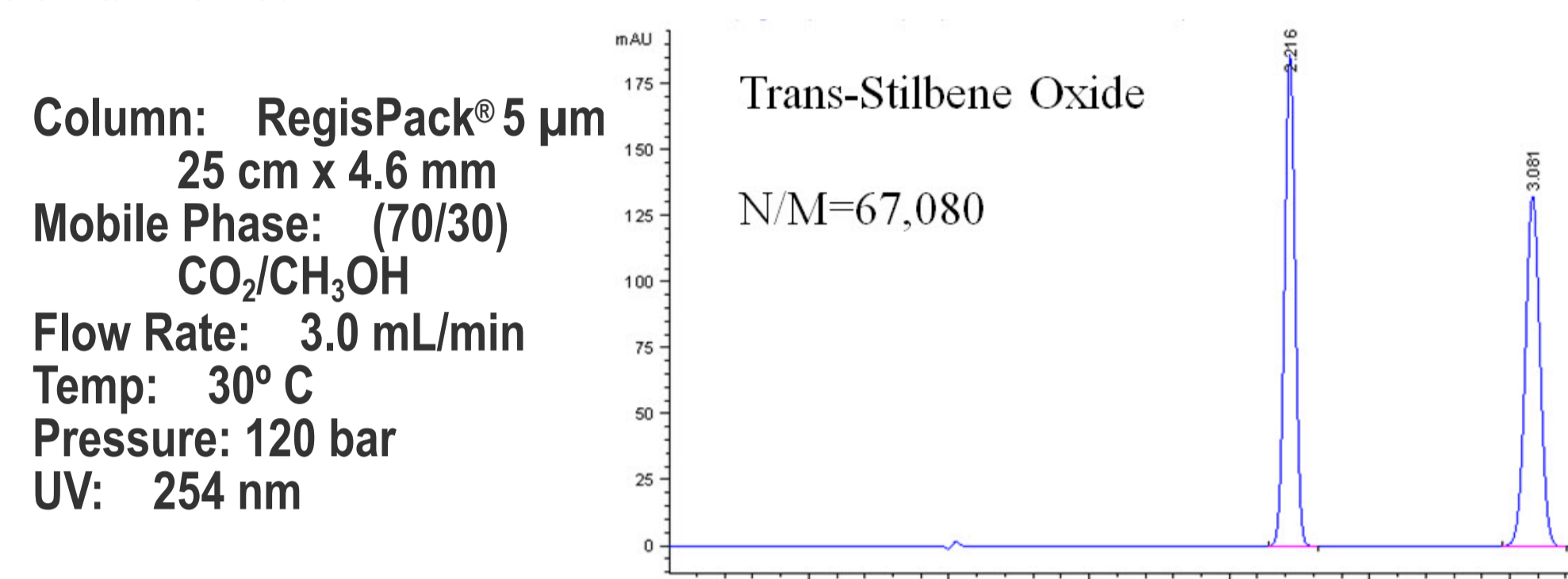
Instrumentation

The instrumentation consisted of a Fusion[™]A5 SFC conversion module (Aurora SFC Systems, Inc., Redwood City, CA) connected to a Model 1200SL HPLC (Agilent Technologies, Waldbron, DE).

The Fusion module, condenses vapor phase carbon dioxide, and boosts its pressure to just under the column head pressure. Since the CO₂ is being distilled just before use, low cost "beverage" grade can be used. After the detector the mobile phase is returned to the module where an ultra low noise back pressure is used to control system outlet pressure. The compressibility compensation term for the left side of the slightly modified Agilent binary pump was set to zero. This pump meters the carbon dioxide flow. The absence of a compression stroke by the metering pump, along with the low noise BPR, produces ultra-low flow and UV detector noise. The diode array detector was fitted with a high pressure 1.7 μL flow cell. A standard ChemStation controls total flow, all injection parameters, column temperature, and detector parameters. A small software add-on is used to control the system outlet pressure.

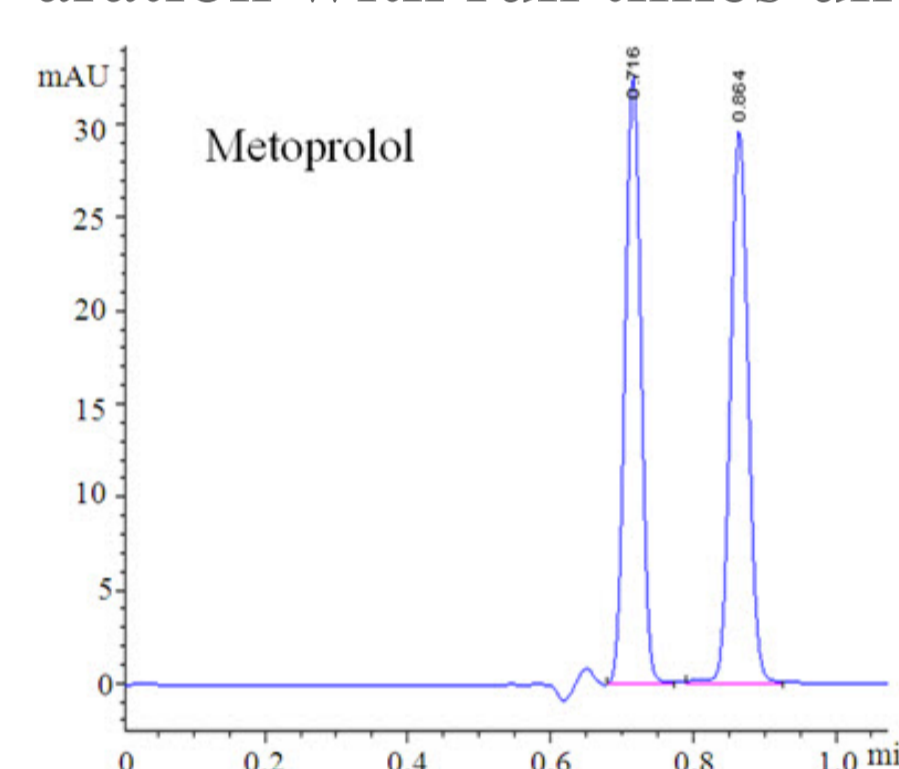
High Efficiencies

Fig 1: Chromatogram displaying high efficiency under SFC conditions.



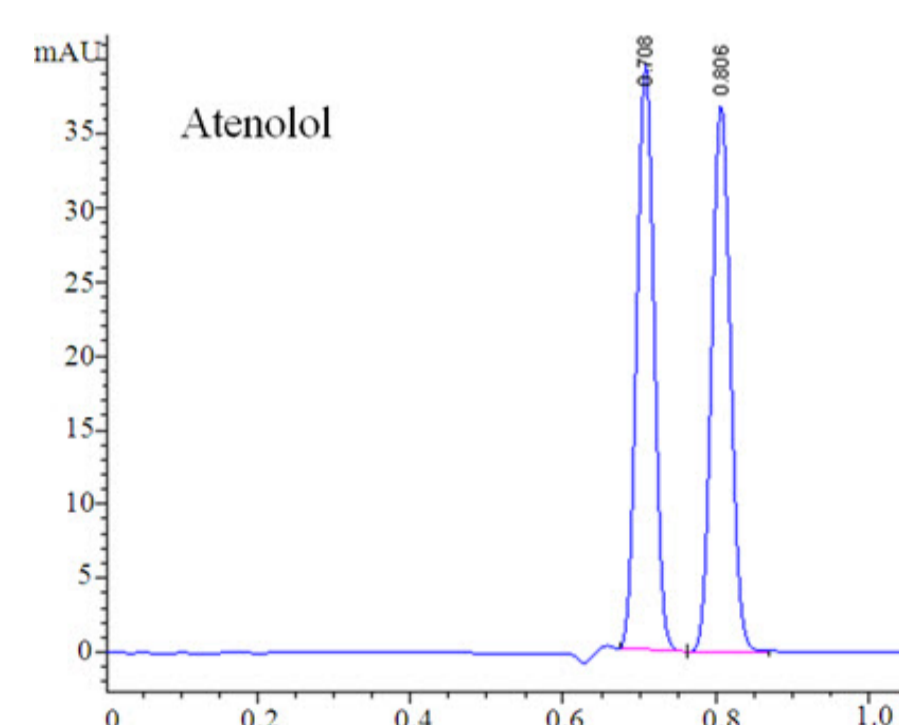
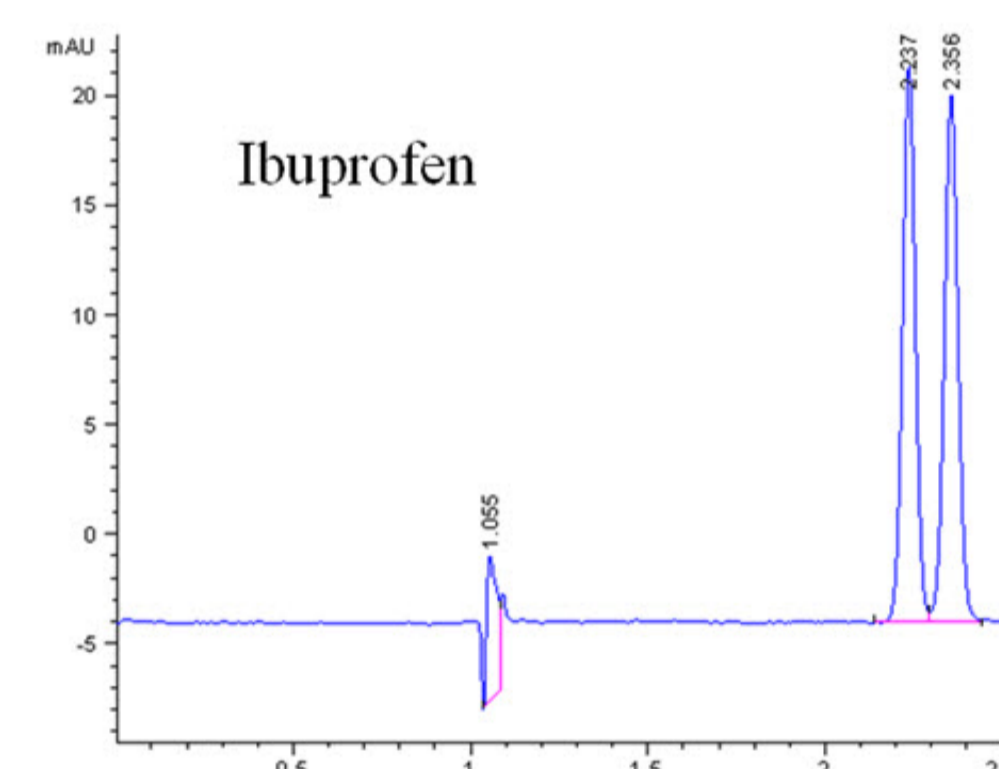
Fast Separations

Figs 2-5: SFC chromatograms demonstrating excellent separation with run times under 2.5 minutes.



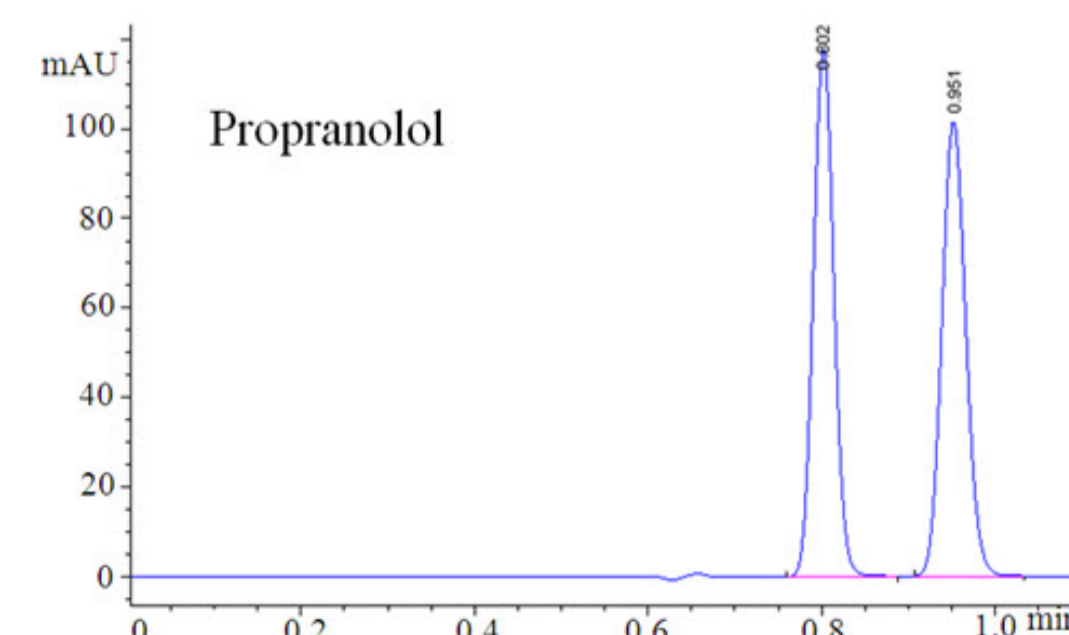
Column: RegisCell[®] 5 μm
25 cm x 4.6 mm
Mobile Phase: (60/40)
CO₂/CH₃OH
+ 0.1% TEA
Flow Rate: 5.0 mL/min
Temp: 30° C
Pressure: 120 bar
UV: 254 nm

Column: RegisPack[®] 5 μm
25 cm x 4.6 mm
Mobile Phase: (90/10)
CO₂/CH₃OH
Flow Rate: 3.0 mL/min
Temp: 30° C
Pressure: 120 bar
UV: 254 nm



Column: RegisCell[®] 5 μm
25 cm x 4.6 mm
Mobile Phase: (50/50)
CO₂/CH₃OH
+ 0.1% TEA
Flow Rate: 5.0 mL/min
Temp: 30° C
Pressure: 120 bar
UV: 254 nm

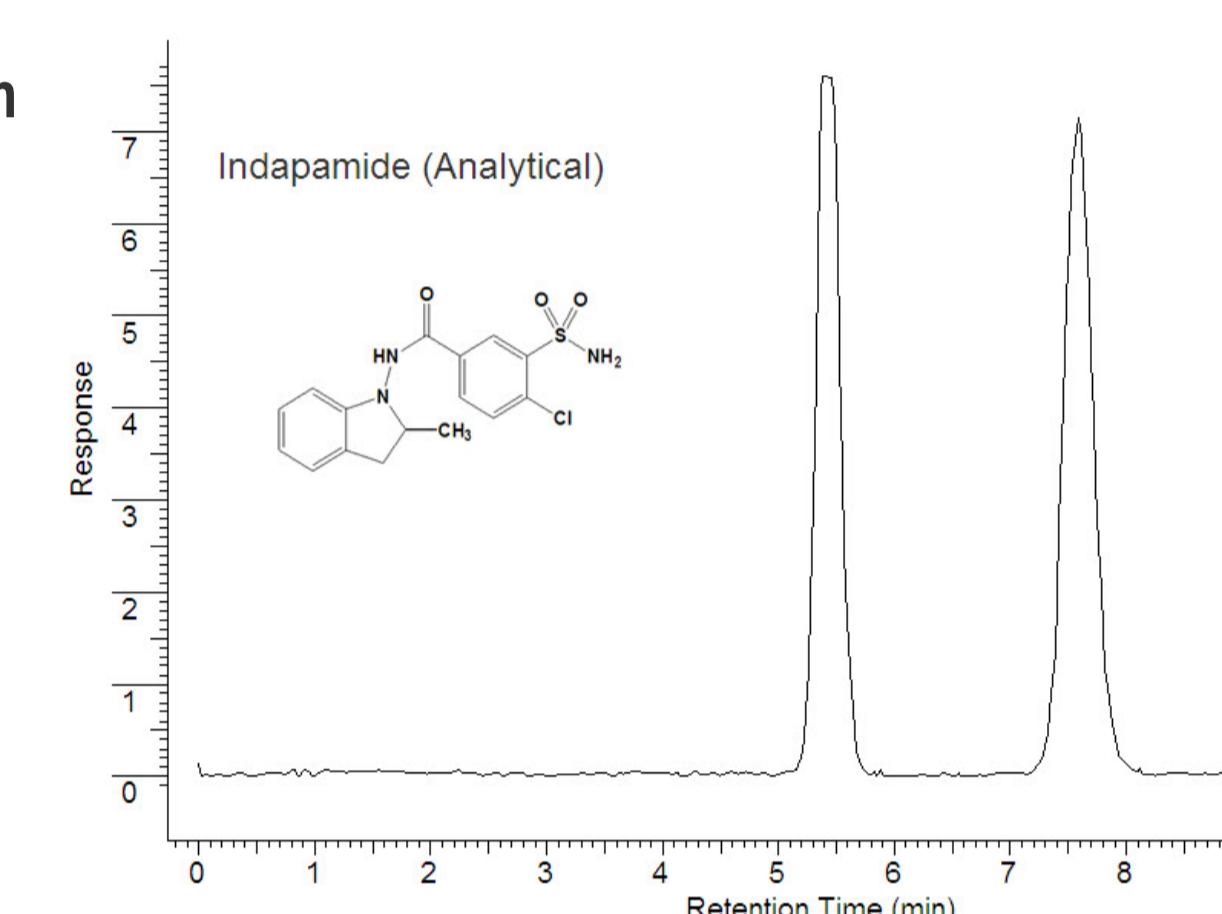
Column: RegisCell[®] 5 μm
25 cm x 4.6 mm
Mobile Phase: (50/50)
CO₂/CH₃OH
+ 0.1% TEA
Flow Rate: 5.0 mL/min
Temp: 30° C
Pressure: 120 bar
UV: 254 nm



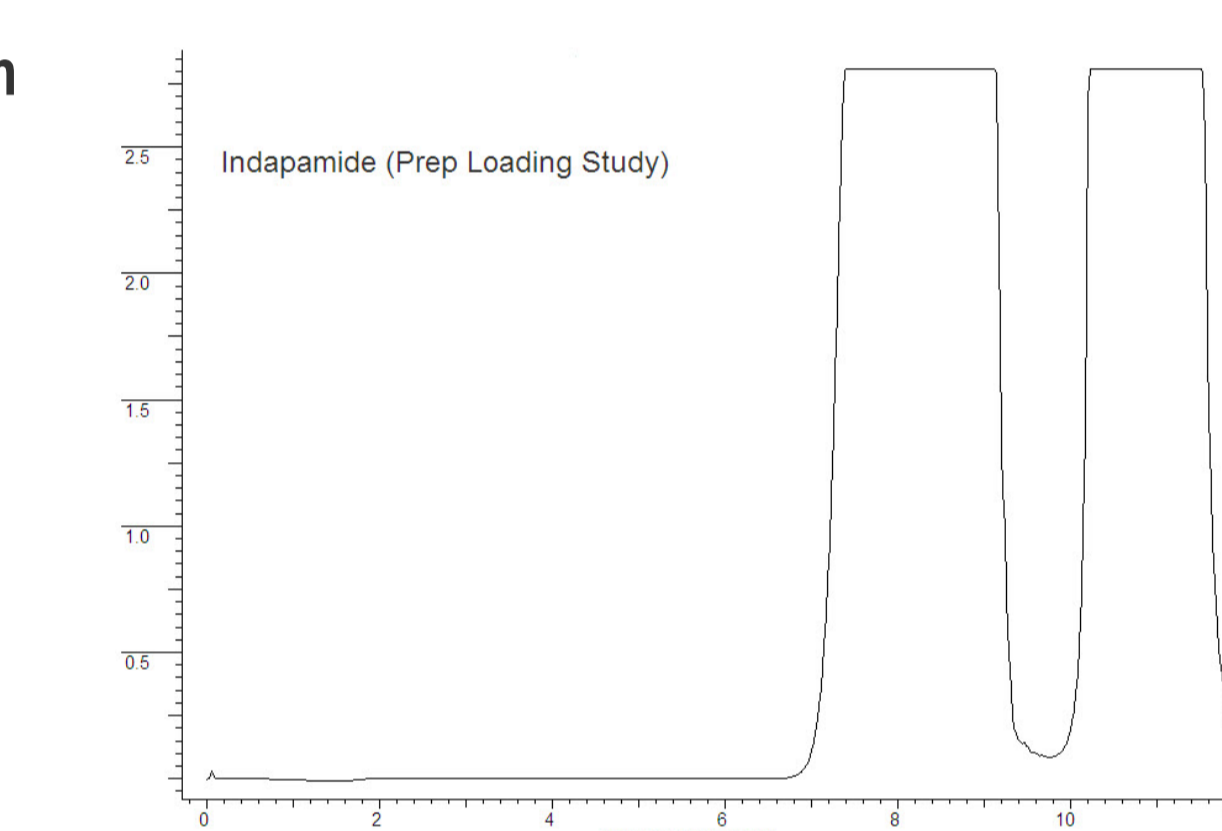
SFC Scale-Up

Figs 6-8: Analytical and preparative methods were used for investigating the suitability of the Whelk-O[®] 1 CSP in its simplicity of scale-up in SFC.

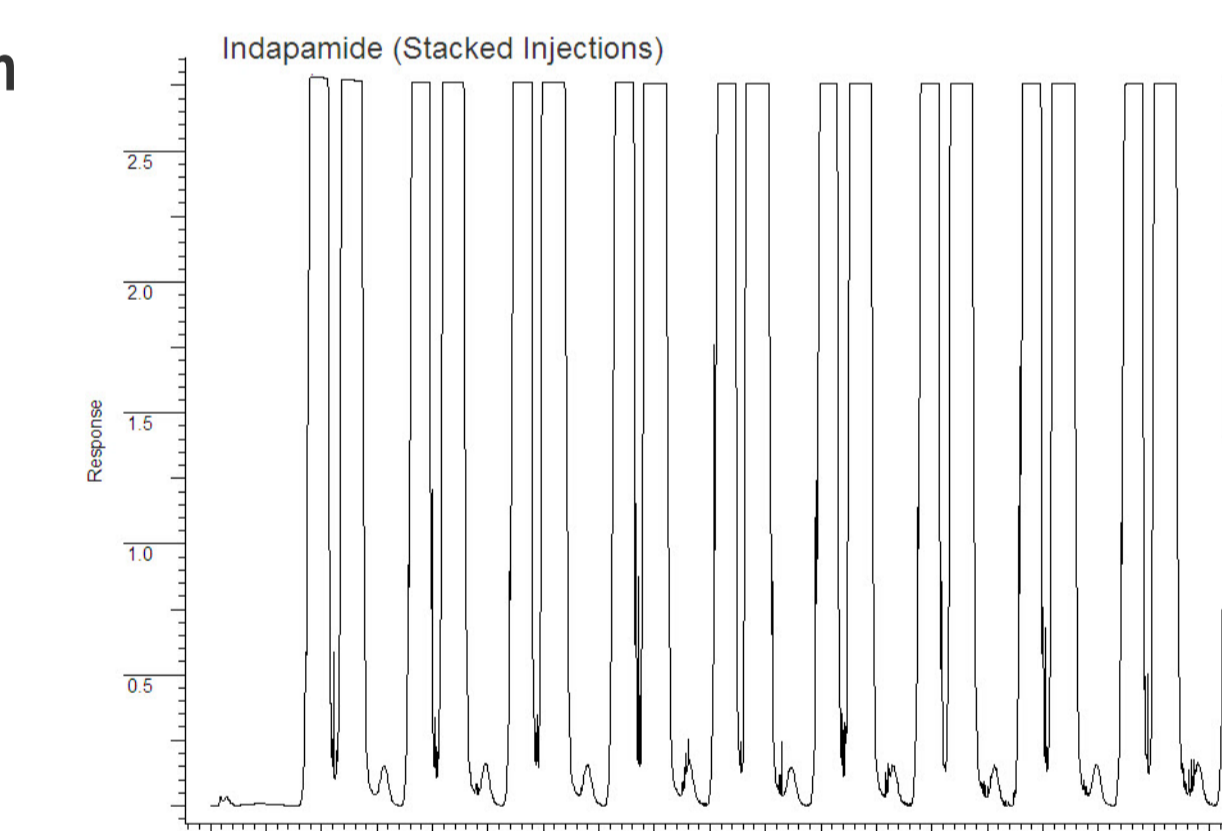
Column: Whelk-O[®] 1 5 μm
25 cm x 4.6 mm
Mobile Phase: (80/20)
CO₂/CH₃OH
Flow Rate: 4.0 mL/min
Injection Vol: 1 μL at
160mg/mL
Temp: 40° C
Pressure: 200 bar
UV: 254 nm
**Instrument: THAR SFC
Method Station



Column: Whelk-O[®] 1 5 μm
25 cm x 4.6 mm
Mobile Phase: (80/20)
CO₂/CH₃OH
Flow Rate: 3.0 mL/min
Injection Vol: 80 μL at
160mg/mL
Temp: 40° C
Pressure: 150 bar
UV: 254 nm
**Instrument: THAR SFC
Method Station



Column: Whelk-O[®] 1 5 μm
25 cm x 4.6 mm
Mobile Phase: (80/20)
CO₂/CH₃OH
Flow Rate: 3.0 mL/min
Injection Vol: 80 μL at
160mg/mL
Temp: 40° C
Pressure: 150 bar
UV: 254 nm
**Instrument: THAR SFC
Method Station



Conclusions

The high efficiencies and rapid separation times generated by the RegisPack[®] and RegisCell[®] columns enable the screening of numerous samples in a short period of time.

Short run times and good sample loading are significant advantages in using SFC for analytical and preparative scale applications. The Whelk-O[®] 1 CSP exhibits high loading and productivity rates for the SFC separation of enantiomers.

The RegisPack[®], RegisCell[®] and the Whelk-O[®] 1 are highly versatile CSP's which offer a comprehensive value added solution to SFC chiral separations.

**RegisPack[®], RegisCell[®] and Whelk-O[®] are registered trademarks of Regis Technologies, Inc.